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Potential methane production of spent sawdust used in the cultivation of *Gymnopilus pampeanus*

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ABSTRACT

Gymnopilus pampeanus (*GP*) is a mushroom consumed in Argentina. Spent *Populus* sawdust (SPS) obtained from the cultivation of *GP* was used to test methane production. The effect of two substrate/inoculums (S/I) ratios was analysed. Three treatments were carried out, T1: 80% SPS + 20% I, T2: 40% SPS + 60% I and T3: 40% PS (*Populus* sawdust) + 60% I. After 105 days the cumulative biogas production resulted in 201.2 and 147.8 mL/g VS for T2 and T1 respectively. Methane production increased 62.2% when S/I decreased 83.3% (112.9 and 71.7 mL/g VS for T1 and T2 respectively) while for treatments which used the same percentage of inoculums (60%) the fungal action on the sawdust improved methane production in 970%. Regarding the kinetics of methane production, Gompertz equation demonstrated a good performance of the adjustment of experimental data ($R^2 > 0.98$) and the values of the kinetic parameters indicated that SPS structure showed better accessibility than PS to the methanogenic system. The long time of adaptation (32.2 days) and the low methane production rate (1.7 mL/g VS d) observed in SPS, revealed that the methane production is still not enough for energy purposes.

1. Introduction

The accumulation of agro-industrial and farming waste represents an important source of pollution, mainly due to the releasing of large amounts of greenhouse gases (GHGs) as well as the harmful consequences for the environment. Mushroom production is an activity that usually uses agro-industrial wastes as substrate. When mushroom production finishes, for every kilogramme of mushroom produced, about 5 kg of spent substrate is generated, which traditionally has been discharged as waste [1]. China, considered as the first mushroom producer country, produced 25,712,000 ton of edible mushroom in the year 2011 and this generated more than 100 hundred millions of tons of spent substrate [2]. This organic material could have added value if it can be transformed to produce clean energy that could be use in the same mushroom farm considering that most of them are located in rural areas where energy supply is not always provided [2].

Anaerobic digestion (AD) is a technological option that simultaneously contributes to mitigate the pollution caused by the inadequate disposal of waste and industrial effluents [3,4] and to provide a source of renewable energy. Many different organic materials can be processed by anaerobic digestion, such as paper [5], sewage sludge [6–8], organic solid [9–11] and animal waste as manure [3,12,13]. Lignocellulosic materials such as woody wastes are exceptions to this behaviour. They are hardly converted to biogas due to their chemical composition and complex structure. While cellulose and hemicellulose can be used by the anaerobic system, lignin however, cannot be degraded under anaerobic conditions [14,15]. In order to increase the biogas potential, treatments that facilitate the access of holocellulose (cellulose and hemicellulose) of bio-fibers are needed [14,16]. Positive effects on the biodegradability of lignocellulosic waste by bacteria have been obtained from physical, chemical or biological pre-treatments improving the production of biogas [14,17–19]. However, physical and chemical pre-treatment have been considered unattractive

Abbreviations: AD, anaerobic digestion; e, mathematical constant (2.718); GHGs, greenhouse gases; GP, gymnopilus pampeanus; M, methane cumulative production (mL/g VS) at time t (d); P, maximum methane production (mL/g VS); PS, *Populus* sawdust; R, maximum methane production rate (mL/g VS/d); SD, standard deviation; S/I, substrate/inoculum ratio; SPS, spent *Populus* sawdust; TS, total solids; VS, volatile solids; λ, lag-phase time (d).

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due to the high costs involved [20]. In contrast, the biological pretreatments have the advantage of low energy requirements and mild environmental conditions [21]. Microorganisms, such as brown, white and soft rot fungi and bacteria can be used as biological treatments to attack the raw material by using their enzymes [15,22]. Between these types of fungi, the genus *Gymnopilus* (Basidiomycetes) has a large number of xylophagous species [23,24]. *G. pampeanus*, also known in Argentina and Uruguay as *G. spectabylis* var *pampeanus* [25], is an edible species that usually grows under *Eucalyptus* trees and that has been recently cultivated to produce fruit bodies for human consumption [26]. For the cultivation of such fungi *Populus* and *Eucalyptus* sawdust are usually used [27–29]. Both are abundant raw material in Argentina since these two types of wood are used to produce furniture and fruit wooden boxes [30]. As a result of the production of this fungus, a large quantity of a spent substrate of low density that requires a large area for final disposal is generated. As this residue is rich in organic matter that has been biologically degraded during mushroom production, it is interesting to evaluate its potential to produce methane by AD.

A wide range of factors affect the production of methane during AD, therefore, the net energy that can be produced by an unknown waste is a complex task. One of the key parameters in a batch high solid AD process is the substrate (S) to inoculums (I) ratio (S/I), expressed as the amount of feedstock volatile solids (VS) added per amount of VS in the inoculum. The amount of inoculum added, as a source of a large number of microorganisms that promote methane production, influences not only the start-up of a batch digester but also determines the ultimate methane yield, as well as the rate of methane production in relation to a potential inhibition of the substrate [31–34]. The effect of inoculum on methane production of different kinds of substrates was studied by several researchers. Chynoweth et al. [35,36] found out that the decrease in S/I may be necessary for recalcitrant substrates and suggested a rate of 1:1 to 1:2. Lesteur et al. [37] reported that S/I between 1:1 and 1:3 are the rate generally used by researchers, although the relationship that optimises the process strongly depend on the substrate used. Cheng and Zhong [33] showed the importance to determine the optimal S/I ratio for unknown substrates in order to minimise the requirement of active inoculum for the start-up of a digester to assure good methane production.

The objective of this work was to evaluate the capability of methane production of the spent substrate of *G. pampeanus* under AD. The effect of two S/I ratios on both, the methane production and the organic matter removal was analysed. The potentially degradable action of *G. pampeanus* on *Populus* sawdust was also analysed regarding the methane production. Experimental data of methane production was modelled through a non-linear model in order to obtain the kinetics parameters of the process.

2. Materials and methods

2.1. *Spent* Populus *sawdust*

The preparation of the substrate used in the experience included the following steps:

2.1.1. Strains used

Gymnopilus pampeanus: ICFC 748/12, Chascomús, Buenos Aires, Argentina; growing on *Eucalyptus*, 04-X-2011, leg M. B. Colavolpe. It is conserved in IIB-INTECH Collection of Fungal Cultures (ICFC), Laboratory of Mycology and Mushroom Cultivation, IIB-INTECH; Chascomús, Argentina (reference in the WDCM database: 826).

2.1.2. Substrate preparation

Populus sp. sawdust was used as the substrate. 25 × 45 cm polypropylene bags were filled with 1000 g of wet substrate mixture with 1% of $CaCO_3$ powder. Moisture was adjusted to 70% using distilled water. Bags were sterilised using an autoclave during 2 h at 120° C and 1.2 psi.

2.1.3. Substrate inoculation

Once bags reached room temperature they were inoculated at 5% w/w in laminar flow with the spawn of *G. pampeanus* which was prepared according to Lechner and Albertó [29].

2.1.4. Substrate fermentation

bags were transferred to an incubation room for 75 days under controlled environmental conditions: temperature 25 °C; humidity 60% and darkness. After this period, bags were removed and the colonised substrates were moved to fruiting room under controlled conditions: temperature 18–20 °C; humidity 80–90% and photoperiod of 9 h light/15 h dark to induce basidiome production. Basidiomes were regularly harvested during 60 days. After this time, blocks were removed from production room, were frozen at −20 °C and considered as spend substrate.

2.1.5. Substrate sampling

Blocks were defrosted at room temperature; four blocks were put in a plastic box; they were disassembled and mixed by hand to obtain a homogeneous sample

2.2. *Inoculum used for anaerobic digestion assay*

Sewage sludge obtained from the local wastewater treatment plant was used as inoculum. In order to ensure the degradation of the easily degradable organic matter that could be still present in the inoculum, it was maintained in a batch reactor at mesophilic conditions at 35 °C ±1 until use [38,39].

2.3. *Experimental design*

Fractional factorials design with two factors each one at two levels was applied (Fig. 1). The factors selected were the percentage of I and the type of substrate. Inoculum was applied at 20 and 60%. The substrates selected were spent *Populus* sawdust (SPS) and *Populus* sawdust (PS). The response variable was the methane production. This design was selected in order to identify the potential degradation action of the fungus on PS and to evaluate the effect of the quantity of inoculum on the methane production.

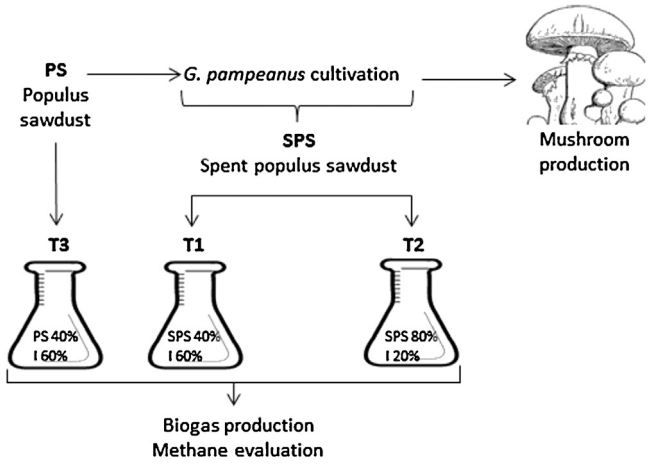

Fig. 1. Experimental setup.